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A HEMOPHILIC BACILLUS FOUND IN URINARY INFECTIONS.*

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WHILE a very great amount of work has been done on the bacteriology of urinary infections it appears that no systematic investigation, at least at all extensive, has been made with blood media. In a series of cases such media was used in the routine examination of the urine and other material from the urinary tract with the result that in three cases an organism was found which apparently can be cultivated only by this method. This organism is here described and an account given of the three cases from which it was obtained together with some data on its specificity.

Case 1. A man, 41 years of age, gave a history of turbid urine for at least 8 years and possibly for a much longer period. Thirty years ago he had some urinary trouble at which time he passed what was probably a small stone through the urethra. Since then he has had no symptoms suggesting calculi. Twenty-five years ago he had an attack of typhoid fever. At the present time he seems to have perfect general health. His nutrition is good and appetite excellent; he has no fever and has worked constantly for years as a salesman. There has always been some tendency to constipation but never marked. He has had no venereal disease. He is married and his wife and several small children are perfectly well. Urination appears to be normal. He is rarely obliged to get up at night to urinate and no symptoms of vesical irritation exist. Physical examination reveals nothing of importance. The left lobe of the prostate is slightly larger than the right; no secretion was obtained by massage. The kidneys are not tender or enlarged. X-ray pictures taken two years ago and at the present time show no evidence of calculi in the urinary tract. A cystoscopic examination made three years ago gave negative findings. Examinations of the urine for tubercle bacilli by the staining method and also by guinea-pig inoculations were without result.

Examination of the blood gave the following: Red cells 4,800,000; hemoglobin 90 per cent; the leukocytes were counted at about weekly intervals for several weeks and always ranged between 10,000 and 13,000. Differentiation gave an increase in the eosinophiles, the percentage of these cells varying from 6 per cent to 12 per cent; the other cells occurred in normal proportions. This eosinophilia led to careful examination of the urine and stools for parasites and ova but none have thus far been found. Possible echinococcus of the urinary tract was especially considered, but a history indicating the possibility of passing the parasite was not obtainable, and repeated examinations of the urine for hooklets, etc., have given negative results. The temperature taken morning and evening for two weeks was never above normal.

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The urine has been examined at frequent intervals for over a year. It was always obtained by having it passed directly into a sterile bottle after sponging the meatus and allowing the first urine to escape, and then immediately examined by smear and culture. It was always very turbid and on standing a voluminous white sediment soon appeared, the urine above becoming remarkably clear. The specific gravity was usually about 1,016 and a slight reaction for albumin and a distinct reaction for mucin could always be obtained. No sugar. The reaction was constantly alkaline to litmus but was never ammoniacal.

Microscopically no casts or red corpuscles were ever found. Polynuclear leukocytes were always present in large numbers and usually in a very good state of preservation. It was rare to find mononuclear or epithelial cells. Counts of the leukocytes in the urine made at intervals of one or two weeks for six months ranged from 1,200 to 4,000 per cu. mm. The bacilli which will be described later were always found in large numbers both within and without the leukocytes. Examinations have been made at short intervals for over a year and the case is still under observation. As a rule no other organisms have been seen in smear or culture. In two or three instances in smear preparations a few gram-positive diplococci were observed. They were not cultivated and were not constantly found. As these specimens were not obtained by catheter it is possible that they came from the urethra.

Case 2. This patient, a young woman, gave a history of having had, in April, 1908, an indefinite "grippe" infection which lasted four weeks and after which she did not properly regain her strength. Two months later she was exposed to cold and dampness and on the second day following, she noted stiffness in the left knee joint which soon became painful when she put her weight on it. One week later the other knee became similarly affected. Slight swelling occurred about the joint. She remained in bed for six weeks without perceptible improvement and during this time she also had pains in the elbows and finger joints which were not severe and of short duration. It was for this trouble that she entered the Presbyterian Hospital in the service of Dr. Billings in October, 1908. At this time no appreciable swelling of the joints was noted altho she complained of stiffness and pain on motion. The temperature occasionally rose to 99°; no chills. The red corpuscles numbered 4,846,000; the white corpuscles ranged from 10,000 to 15,000. At no time were definite urinary symptoms complained of. In a catheterized specimen of the urine, however, were found leukocytes and a small bacillus. The bacillus was cultivated and found to possess the cultural characteristics of the bacillus in Case 1. A vaccine was prepared and inoculations begun and continued weekly for nearly three months. Catheterized specimens of the urine were obtained each week for several weeks. They were always slightly turbid and contained a few polynuclear and epithelial cells but no red cells or casts. The leukocytes in number ranged from 20–50 per cu. mm. of urine. At times a small amount of serum albumin was found; and quite constantly there was a distinct reaction for nucleo-albumin; no sugar. The reaction to litmus as a rule was slightly alkaline. In every specimen there was found in smears and culture the small bacillus referred to; no other bacteria were present. They were much less numerous than in Case 1 but in the centrifuged sediment they were easily found, usually in small clumps about the leukocytes and the few epithelial cells commonly found. One drop of urine per plate gave in the first specimens examined from 20 to 40 colonies.

Case 3. This patient, a man 39 years of age, was in the service of Dr. Billings in the Presbyterian Hospital. He commenced to have spinal trouble about eight years ago. He first noticed pain in the neck on turning the head and soon the pain extended downward involving the thoracic spine. Associated with the pain was spinal rigidity and deformity which advanced to a well-marked kyphosis. Three years ago he commenced to wear a brace which relieved the pain greatly and for the past two years he has had little or none and the deformity has not advanced. At the present time the entire spine is held rigid and motion in all directions is very much restricted. Slight crepitus is heard on rotation of the neck. Other joints of the body do not appear to be involved at present and have not been affected in the past. For many years he has had recurrent attacks of tonsilitis, two of which occurred before the spinal trouble began. These attacks are severe but of short duration. At present the tonsils are slightly enlarged and the surfaces irregular but are not acutely inflamed. The throat is normal. The tonsils were removed and in the crypts was a nearly pure culture of streptococcus pyogenes. Thirteen years ago he had an attack of gonorrhea and has also in the past had attacks simulating asthma. Four years ago he had cystitis the nature of which was undetermined. At present his general health is good, and physical examination, excepting the findings given above, reveals nothing significant. The prostate is slightly enlarged and firm, and massage expresses a small amount of a thin discharge in which are leukocytes and intracellular gram-negative organisms suggestive of gonococci. They have not been cultivated. He complains of no urinary symptoms at present. The urine (catheterized) is clear, acid in reaction, contains no albumin, sugar, or casts, but an occasional leukocyte can be found. In blood agar plate cultures and in smears of the sediment were found a considerable number of small gram-negative bacilli which grow only on blood media and in every respect correspond to the organism from Cases 1 and 2. No other bacteria were found.

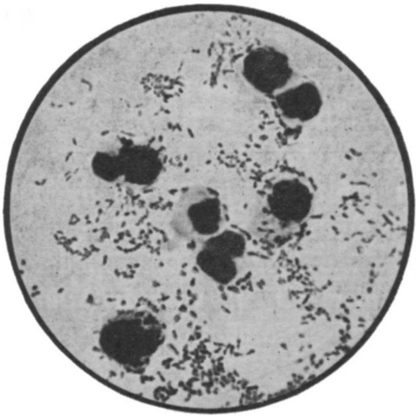

FIG. 1.—Hemophilic bacillus from urine. Smear preparation of urinary sediment from Case 1. ×1,000.

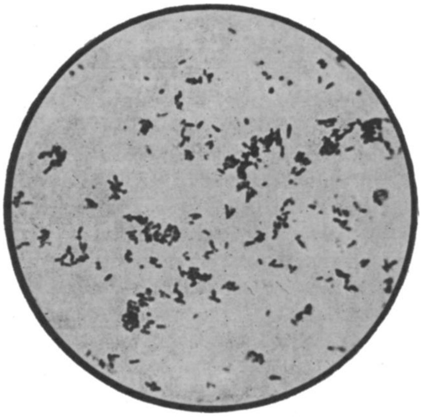

FIG. 2.—Hemophilic bacillus from urine. Pure culture three days from blood agar. ×1,000.

Morphology.—The organism is a small non-motile, gram-negative bacillus whose size varies considerably but is usually about that of the influenza bacillus. At times, and especially in the urine, it is distinctly larger and may be quite plump and short. Its measurements may be given at from 0.2–0.3 μ.×0.5–0.8 μ. It stains readily with ordinary dyes and is non-acid and non-alcohol fast. With methylene blue it often stains unevenly, sometimes more deeply at the ends, and again fairly distinct granules may be made out so that it looks not unlike very minute diphtheria bacilli. There is no tendency to form threads or chains, and a characteristic grouping has not been made out tho it is common to see the organisms both from urine and from cultures arranged in small irregular clumps consisting of several bacilli. In the urinary sediment they may be seen clustered about the pus cells, probably having been thus carried in the process of sedimentation. They occur both within and without the leukocytes and at times seem to adhere to epithelial cells as was observed especially in Case 2. The bacillus does not form spores and does not possess a capsule.

Cultural Characteristics.—The rather remarkable cultural characteristics of this organism first attracted my attention. In Case 1 the large number of small bacilli were noted in the smear preparations, and when aerobic blood plates were made no growth appeared. From a second specimen similar plates were made and placed under aerobic and anaerobic conditions. In the latter in 24 hours definite small colonies were seen, evident to the naked eye chiefly on account of the clear, wide zone of hemolysis surrounding them. In this instance, too, much smaller tho similar colonies were seen on the aerobic plates after 24 hours. As a rule the bacillus grows more luxuriantly and more quickly under anaerobic conditions and failure to grow under aerobic conditions is occasionally noted on blood plates. Growth always takes place slowly and continues for several days. This is more noticeable on blood agar slants where often no growth is evident to the naked eye in 24 hours. At the end of 48 hours, as a rule, very delicate growth is manifest in the form of tiny, discreet, dark grey colonies. It continues to increase slowly until the fourth or fifth day, after which no further growth is apparent. It never becomes profuse and may require the aid of a hand lens to be seen at all. It is slightly raised above the surface, is easily removed with the loop, and is not tenacious or slimy. This organism does not grow at room temperature. On the plates the colonies as a rule in 24 hours are grey, almost invisible to the eye, and the clear zone of hemolysis measures from 0.5 to 1 mm. in diameter. There is no tendency for the growth to spread on the surface. Under the microscope the colonies are finely granular, grey or greyish brown, the margins are regular and about the colonies the red blood corpuscles have entirely disappeared. The outer margin of the zone of hemolysis is usually quite definite, being in this respect similar to the zone about streptococcus colonies, altho often it may be seen to shade more gradually into the surrounding media. The zone of hemolysis is not colored but often is slightly hazy. The colonies present the same appearance on plates with human, rabbit, goat, and pigeon blood. Attempts to grow this bacillus on media not containing blood have persistently failed. Plain agar, glycerin agar, ascites agar, Löffler's blood serum, lecithin agar, potato, gelatin, broth, milk, and sugar media give no growth under either aerobic or anaerobic conditions. When a few drops of blood, either human or animal, are added, growth occurs. Fluid blood media are not as favorable as solid blood media. Milk and various sugar broths to which blood has been added show no change or fermentation. Attempts to grow it in alkaline and acid normal urine pure or when added to agar or broth have failed. This was somewhat surprising in view of the fact that the bacilli in the body develop in urine which apparently is free from blood. Filtered patient's urine and filtered nephritic urine give no growth. Plain media made so as to contain varying percentages of acid and alkali likewise gave negative results. In Libornius tubes good growth was visible in blood media but never obtained when ascites, sugar, or other media not containing blood were used. Strains of the bacilli from the three cases have been cultivated side by side on the various media and no noteworthy differences have been observed at any time. The organisms from Cases 1 and 2 have been grown on blood agar slants now for several months, transfers having been made every four or five days or oftener, and the growth is the same in appearance and the bacilli have the same morphology as when first isolated. Growth is possibly slightly more profuse after long cultivation and the bacillus shows no tendency to die out as was observed when first isolated. Several times in recently isolated cultures growth in subculture was not obtained after three or four days. As a rule the viability is much greater. In one blood agar tube, kept moist and at room temperature, the bacilli grew at the end of 12 days, 18 days, 23 days, but not after 30 days. In the patient's urine (Case 1) they die much more quickly. A specimen of urine was kept in the ice-box, plate cultures being made from day to day with definite amounts of the urine. Plates inoculated with four drops of the urine gave approximately 1,200 colonies immediately after voiding; after 24 hours approximately the same number; after 48 hours there was a decrease to about 800 per plate; after 72 hours about 100 colonies per plate; after 96 hours only a few colonies appeared on the plate and after 120 hours none were found. A similar experiment, leaving the urine at room temperature, gave approximately the same result. There was a gradual decrease in the number of colonies each day and at the end of five days the urine was sterile. Heating to 55° C. for 10 minutes suffices to kill this organism when suspended in salt solution.

Pathogenicity.—For animals the bacillus has little or no pathogenic power. Inoculated subcutaneously abscess formation does not occur in rabbits or guinea-pigs. Intraperitoneal injections in very large doses may cause death in rabbits in 24 hours and the organism may be recovered from the peritoneal fluid. They have not been recovered in the heart's blood or in other fluids. Intravenous injections in rabbits gave no result.

Phagocytosis.—In the urine there is always a considerable amount of phagocytosis apparent tho the amount may be variable. At times 50 per cent of the leukocytes contain bacilli and again not more than 10 per cent contain them. It is common to see the bacilli clustered about the leukocytes as if adhering to the surface. In vitro they are readily phagocytable in serum and to a certain degree spontaneously. In Case 1 opsonic indices were taken at about weekly intervals for two months and during this time injections of the dead bacilli were given. Beginning on June 19 the opsonic indices were as follows: June 19, 1.6, June 24, 4.5, July 6, 6.4, July 19, 4.9, July 21 3.0, July 29, 2.6, August 8, 1.6, August 25, 2.8. On June 24 inoculations with the dead bacilli were begun and continued about every 10 days for three months in doses of about 150 million at first and gradually increased to one billion. Marked local reaction occurred with each injection and after the larger doses some general reaction manifested by slight rise in temperature, feeling of depression, some muscle soreness but no bladder irritability. The reactions were never severe. Examination of the urine from time to time during the period of inoculations and also after the injections were discontinued revealed no appreciable change with respect to the number of bacilli or to the amount of pus present.

Case 2 was also given injections with the dead bacilli each week for a period of eight weeks in doses of from 100 million to 600 million. Slight local tenderness always developed and as a rule there was slight rise in temperature and some increase in leukocytes in the blood but no vesical irritation. During this period the number of bacilli in the urine decreased but they did not entirely disappear. The pus cells also became less, and in one or two specimens were absent, tho later they appeared again in small numbers. The patient complained less of the pain and stiffness in the joints tho probably little stress should be placed upon these subjective symptoms as she is of a neurotic temperament. Before the injections were given the opsonic index was 0.66. Later after several were given it was 1.8. Some spontaneous phagocytosis occurred.

Agglutination.—In Case 1 the serum was tested for the presence of agglutinins, at six different times, twice before the inoculations were begun and again later. Tests were made both macroscopically and microscopically in normal and patient's serum and at no time was there distinct evidence of specific agglutination. In dilutions of 1:10, 1:20, and even 1:40 in both normal and patient's serum it was common to note microscopically clumping of the bacilli into small masses of 6 to 10 or more, but this was also seen in salt solution. The serum from Case 2 was also tested with negative results. A rabbit was injected 15 times about once a week with living cultures and from time to time the serum was tested for agglutinins but always with negative results. An opportunity was not afforded to test the serum in Case 3.

Bacteriolysis.—Suspended in normal and patient's serum using various dilutions with salt solution this bacillus shows no evidence of lysis either microscopically or by the plate method. After 18 hours there was a slight reduction in the number of colonies on the plates which was about the same in normal and in the patient's serums unheated and heated. No increase in the number of colonies takes place when the suspensions are incubated. Rabbit's serum in small amounts failed to show any evidence of reactivation of heated (56° 30 min.) patient's (Case 1) and normal serum.

Complement Deviation.—In Table 1 are given the results of an experiment to determine the presence of specific antibodies in serum by the method of Bordet and Gengou. The results are those obtained after one hour's incubation. It will be noted that with patient's serum (Case 1) and immune rabbit's serum (mixtures 1 and 3) the hemolysis was not completely inhibited, but compared with the normal sera (mixtures 2 and 4) greatly retarded. The experiment was made using varying amounts of serum (from 1 to 4 drops per tube) and also using varying amounts of antigen both living and

TABLE 1.
COMPLEMENT DEVIATION.

One Hour at 30° C.					Hemolytic System	Result Hemolysis
Mixtures	Serum 2 Drops	Complement (Guinea-Pig)	Antigen	Salt Solution		
1.....	Patient's Serum	.04 c.c.	2 drops dense living suspension	1 c.c.	Suspension red corp. 1 c.c.+ 1/125 c.c. amboceptor	Trace
2.....	Normal "	"	"	"	"	Almost complete
3.....	Immune rabbit "	"	"	"	"	Trace
4.....	Normal rabbit "	"	"	"	"	Almost complete
5.....	Patient's "	"	....	"	"	" "
6.....	Normal "	"	....	"	"	" "
7.....	Immune rabbit "	"	....	"	"	Marked
8.....	Normal rabbit "	"	....	"	"	Almost complete
9.....	......	"	....	"	"	Complete
10.....	......	....	....	"	"	None

heat-killed. The results were similar in practically all the tubes except in the set of tubes containing the dilute antigen (1 drop of 1:3 and 1:5 dilution) in which case there was little or no evidence of inhibition of hemolysis. The living and dead antigen appear to act much alike, the dead antigen possibly being less favorable.[1]

Having observed this organism in the urine in three cases the question arises as to the frequency with which it occurs in pathologic conditions of the urinary tract and also as to whether or not it occurs at times normally. Since this bacillus was first found in Case 1, specimens from about 50 cases of urinary infections in men and women have been examined by the blood agar plate method with especial reference to this organism, and the two additional cases were found among this number. It might, therefore, seem that these bacteria may not be exceedingly rare if proper examinations are made to detect them. More examinations must be made to determine this, however. With a view of determining whether or not they occur in normal urine, catheterized specimens were obtained from women nearly all of whom were gynecological patients in the Central Free Dispensary of Chicago. Cases presenting evidence of infection of the urinary tract are not included in this group. In 43 cases[2] the urine was obtained under sterile precautions by catheter and immediately taken to the laboratory for examination. In all cases both aerobic and anaerobic blood agar plates using 1 c.c. of urine were made and examined from day to day for five days for bacteria. The urine was centrifuged also and the sediment examined for leukocytes, casts, etc., and in stained smears for bacteria. In 38 cases the plates remained sterile and nothing of importance was found in the urinary sediment. In the remaining cases bacteria were found in smears and culture but no pus cells or other urinary sediment. In two cases the colon bacillus occurred in pure growth but few in numbers. In one a few colon bacillus colonies appeared on the plates and in the sediment they were associated with a gram-negative coccus which did not grow in the cultures. In the remaining two were found gram-positive bacilli in small numbers which appeared on the plates, grew on ordinary media and belonged to the pseudo-diphtheria group. In no instance did I find a bacillus similar to the organism now described.

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[1] I am indebted for the hemolytic serum to Dr. W. E. Post, who at the time was using it for Wasserman tests which had been proved thoroughly trustworthy.

[2] These cases were women who came to the dispensary for treatment and include chiefly diseases of the uterus and its appendages. I am indebted to Dr. Heaney and Dr. Rasmussen for aid in obtaining the specimens.

Concerning the significance of this organism in urinary infections definite statements at the present time perhaps may not be made. It is probable that like the colon bacillus it may find suitable conditions for its development produced by other primary infections or by mechanical processes. It evidently is not highly pathogenic.

The literature on the bacteriology of urinary infections is very extensive and the most important works have been consulted carefully to determine whether an organism corresponding to the one I have described has been observed before. As previously stated nearly all of the cultures have been made with non-hemoglobin media, which are quite worthless in isolating this organism. This is true, for instance, of the extensive work of Rovsing, Melchior, Faltin, Brown, Raskai, Suter, Tanaka, and others, which includes many hundreds of cases. There is an occasional reference to cases in which organisms were found in smears in the urine but were not cultivable. Scant attention has been given to these and it is not unlikely that some of these cases may have been infections with this hemophilic bacillus.

The fact that this bacillus appears to require hemoglobin for its continued growth suggests at once its relationship to Pfeiffer's bacillus (B. influenzae). While in this respect it is similar, in other features it is so strikingly different that there is little reason for confusing the two organisms if proper tests are made. Briefly the differential points are as follows: My bacillus is always hemolytic on blood agar plates; the influenza bacillus does not hemolyse. The influenza bacillus shows the phenomenon of symbiosis; this bacillus does not. The influenza bacillus grows rapidly, requires only a very minute amount of hemoglobin and shows a distinct preference for pigeon's blood. This bacillus grows slowly, requires relatively large amounts of blood in the media and shows no preference for pigeon's blood. The colonies of influenza bacillus as a rule are distinctly larger and are more transparent and "dew drop" like than are the colonies of this bacillus, which are very minute and more opaque. Morphologically the influenza bacillus is more uniform in size and outline and in older cultures tends to produce thread forms, which this bacillus never does.

In the literature are a few cases in which Pfeiffer's bacillus is reported to have been found in the urinary tract and the question arises as to the possibility of the organisms from some of these cases having been confused with the bacillus here described. Cohn,[1] in a case of acute urethritis, found in smears and culture of the purulent discharge a bacillus in mixt growth with another encapsulated bacillus which Neisser identified as B. influenzae. It grew only on hemoglobin media, preferred pigeon blood and in other respects corresponded with Pfeiffer's bacillus. Later cystitis developed but no statement is made whether or not the bacillus was found in urine from the bladder. J. H. Wright[2] found in mixt culture in a case of pyelonephritis associated with a stone a bacillus which culturally and morphologically agreed with B. influenzae. Paltauf and Kretz[3] found the influenza bacillus in the kidney post mortem. From the descriptions given in these reports there is probably little doubt but that the organisms were B. influenzae. At any rate they are distinctly different from the bacillus here considered. It is of interest to note that we may find in the urinary tract at least two kinds of hemophilic bacilli which without careful differentiation may be confused. Friedberger[4] found in the preputial secretion of dogs a bacillus- "B. hemoglobinophilus canis" which resembles this bacillus in some respects more closely than does B. influenzae. It is a gram-negative, non-motile, small bacillus requiring hemoglobin for growth, and like our bacillus does not manifest symbiosis. However, it is differentiated by its failure to grow under anaerobic conditions, its uniform staining, and by the fact that it attains its maximum growth in 24 to 30 hours. Wolff[5] described an organism isolated from a rat which was hemophilic and gram-negative but somewhat larger than the influenza bacillus. After three months' cultivation on artificial media it acquired the property of growing on non-hemoglobin media. Our bacillus after four months cultivation has shown no such tendency.

Attention has been called to the fact that the bacillus at times bears some resemblance to forms of B. diphtheriae and its possible

[1] *Deutsche med. Wchnschr.*, 1905, 31, p. 1152.

[2] *Boston Med. and Surg. Jour.*, 1905, 152, p. 496.

[3] Quoted in *Modern Medicine*, 2, p. 482.

[4] *Centralbl. f. Bakt.*, 1, 1903, Orig., 33, p. 401.

[5] *Ibid.*, 1, 1903, Orig. 33, p. 407.

relationship to this group should be thought of. Pfeiffer[1] in an extensive article on the bacteriology of the urethra found pseudodiphtheria bacilli in 83.7 per cent of the cases examined. He divides the strains isolated into two groups, one in which growth is scant and a second in which growth is much more luxuriant. The bacilli of the first group remind one of our bacillus inasmuch as he states that at times on plain agar it does not grow, but grows well, tho slowly, on serum agar, which media he used to isolate it. However, the bacillus is much larger, being $0.3\ \mu$ to $0.8\ \mu$ wide and $2\ \mu$ to $3\ \mu$ long, is gram-positive, grows on non-hemoglobin media, and grows well on milk, which it acidifies. His second group corresponds to the more common pseudodiphtheria bacillus, which has little resemblance to our bacillus. Pfeiffer, however, isolated a small bacillus in one case which may very well be identical with our organism. It was a minute, gram-negative bacillus very similar, he states, to B. influenzae, and its colonies were small and finely granular. It grew on his beef serum medium but not on plain media and died after the third generation. Inasmuch as serum practically always contains some hemoglobin its growth may thus be explained. His description is very brief and he refers to it as an influenza-like bacillus. It is of interest also that in the literature occasionally one meets with statements that influenza-like bacilli were seen in smears of secretions of the urinary tract which did not appear in cultures. It is not unlikely that careful cultural work with blood media will reveal the nature of such bacteria.

SUMMARY

A bacillus has been isolated in pure culture from the urine in three cases. It grows only on media containing hemoglobin but differs in many important features from B. influenzae. Two of the cases had pyuria and the third gave a history of urinary infection some time previously. Two of the cases (Cases 2 and 3) had joint trouble but there is no evidence to indicate a causal relationship between the bacillus and the joint affection. There is reason to believe that this bacillus may not be rare and that it has been overlooked because of its hemophilic property.

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[1] *Arch f. Derm. u. Syph.*, 1904, 69, p. 379.